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BRIEF REPORT

Preweaning Body Marking Reduces Brain Mast Cell Numbers in Rats[1]

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In a spilt-litter design, rats *(Rattus norvegicus)* from four litters between 5 and 20 days of age were given either electric foot shock or no shock daily. Identification of treatment was maintained by daily applications of a permanent marking pen to the dorsal rear body surface and was counterbalanced for treatments across litters. Eight 15- and 20-day-old brains from rats that had been marked, regardless of shock or no shock treatments, displayed a significant ($P < 0.01$) 50 to 70% reduction in mast cell numbers within diencephalic tissue relative to eight non-marked littermates; a similar effect was noted in the adjacent leptomeninges ($P < 0.001$). The shock treatment did not significantly influence MC numbers. Compared to 15-day-old brains, 20-day-old brains displayed a significant ($P < 0.05$) increase in parenchymal MCs, a significant ($P < 0.001$) decrease in adjacent leptomeningeal MCs, and a marginally significant ($P = 0.05$) reduction in total diencephalic MC numbers.

Mast cells (MCs), known to contain histamine, heparin, and other bioactive substances, have been reported in the rat brain by several investigators (Dropp, 1972, 1976; Krüger, 1974; Ibrahim, 1974; Persinger, 1977). Calculations (Dropp, 1976; Krüger, 1974) and recent experimental evidence (Schwartz, 1975) indicate that MCs could account for the total histamine measured in the young rat brain and for a significant portion of this amine's concentration in the adult rat brain. Predictably, the age-dependent changes in whole-brain histamine are reflected by brain MC numbers. MCs, which are found within the meningeal fabric and CNS parenchyma (predominantly around blood vessels), have been implicated in: (1) local hemodynamics, (2) reactions against endogenous/exogenous toxins, and (3) immune-related activities (Persinger, 1977). Brain MCs in adult rabbits, and to a lesser degree in rats, have been reported to degranulate and to release their biopotent substances following injections with compound 48/80 or hydrocortisone or exposures to ionizing radiation (Ibrahim, 1974; Ibrahim, *et al.*, 1976).

Extremely large numbers of mast cells are found in the rat brain before

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weaning. Unpublished data from this laboratory indicate that mean numbers of MCs per 10 μm in the cerebrums of 10 (n = 8)-, 15 (n = 16-, and 20 (n =16)-day-old rats were 89.9 ± 16.3, 53.2 ± 25.4, and 35.1 ± 15.9, respectively, while the mean numbers of MCs per 10-μm section in diencephalic parenchyma (where a majority of MCs occurs in the adult brain), were 0.8 ± 0.7, 10.3 ± 8.6, and 20.0 ± 10.0, respectively. Considering the potential importance of brain MC function, the question was raised whether different forms of early experience could significantly alter brain MC numbers. Two treatments, electric foot shock and body marking, were selected to test this possibility, since their early applications have been demonstrated many times to affect profoundly both histamine-related processes (Stein *et al.*, 1976) and emotional behavior (Denenberg and Haltmeyer, 1967; Newton and Levine, 1968) in the rat. The latter effect was considered important in light of the tendency for MCs to cluster in thalamic nuclei associated with tactual/proprioceptive inputs and the Papez circuit.

Five-day-old first litters (eight pups per litter) from four 120-day-old albino Wistar rat *(Rattus norvegicus)* mothers were recruited into a split-litter design, routinely used by this experimenter (Persinger *et al.*, 1976) for testing early experience paradigms. Half of the pups in the four litters were given 1.5-mA electric shocks to the four feet and ventral surface for 2 min daily on 0.5-cm bars separated by 1.5 cm (LaFayette A-586 one-way shuttle system). During shock delivery, the pups were ambulatory and vocal. The littermate controls also were taken to the shock room, handled, and placed on a similar apparatus for the same period, but not shocked. In order to discriminate subjects receiving either shock (S) or nonshock (NS) treatments within the same litter, the rats were also either: (1) marked (M) once daily for 5 to 10 sec with a Sanford's No. 1000 blue "magic" marker (solvents: xylene–toluene, 90% of volume, + creosol, macrolex blue, and a resin) after receiving the shock/nonshock treatments or (2) not marked (NM). The mark covered approximately a 2.2-cm^2 area of the rear dorsal body surface until the subjects were 14 days old; during this period no obvious skin irritation was noted. (However, no skin histology was performed.) After this age, because of normal body-hair thickness, only the upper one-third of the tail was marked. Marking was alternated in each litter such that S and NS groups had equal numbers of marked and nonmarked subjects. Consequently, there were four experimental conditions: MS, MNS, NMS, and NMNS. All treatments began when the pups were 5 days of age; pups were handled as similarly as possible. In order to attenuate possible ambient artifacts from a single-series procedure, two litters had been delivered 10 days after the first two litters.

Eight littermate female pairs (n = 16) (one marked, one not marked; hence, one shocked, one not shocked) were killed by decapitation on

postnatal Days 15 or 20 (10 and 15 days of treatments, respectively) between 15:00 and 16:00 hr; the time since the last treatments was 24 hr, in order to attenuate possible acute effects. The brains were quickly (2 min) removed with minimal mechanical deformation and the cerebrums were fixed in either formalin (10% formaldehyde; 10%, 10% aqueous CaCl) or EFA[2] (90 parts 80% ethanol, 5 parts 30% formalin, 5 parts concentrated acetic acid); fixatives were counterbalanced for treatments. MS, MNS, NMS, and NMNS brains from each kill were processed together. Following paraffin embedding, brains were microtomed into 10-μm sections. Ten slides, equally spaced between the level of the rostral superior colliculus–medial geniculate and of the fornix–septum, were stained from each brain for MCs by the thionine method, after Lillie (Humason, 1972). Since very few MCs occur in cortical or adjacent telencephalic structures (hippocampus, amygdala) at these levels, only diencephalic areas, where the majority of MCs occurs, were measured. Total numbers of MCs, as defined by metachromatic reaction and morphology, for each 10-μm section were determined in: (1) the leptomeninges, by traversing the complete perimeter of the diencephalon, and (2) the diencephalic parenchyma, by covering the entire area with a grid eyepiece, at 400 $\times$. The mean MC numbers for each brain were calculated by averaging the values from its 10 slides.

As reported by other experimenters (Dropp, 1972; Ibrahim, 1974), the majority of MCs in the 15- and 20-day-old rat subcortical areas investigated clustered within the leptomeninges over the thalamus and around blood vessels through many nuclei of the dorsal thalamus, especially the ventral (dorsal portion) nuclei, anterior nuclei, lateral nuclei, and lateral geniculate of the thalamus. Extremely large clusters of MCs (100 to 200/10-μm section) were evident in 15-day-old brains within the ventricular region of the subfornical organ. Occasional clusters were found around the stria medullaris and stria terminalis.

The means and standard deviations for mean MC numbers per 10-μm section from brains of rats that had been marked or not marked, shocked or not shocked, and 15 or 20 days of age when killed are presented in Table 1. According to three-way analyses of variance (ANOVAs) performed by computer, using SPSS programs, on MC numbers in (1) the leptomeninges, (2) the diencephalic parenchyma, and (3) both areas, marked rats displayed significantly ($F = 12.44$, $df = 1, 8$; $P < 0.01$) fewer MCs in the parenchyma than nonmarked rats, while the 20-day-old rats displayed significantly ($F = 7.68$, $df = 1, 8$; $P < 0.05$) more MCs in this region than the 15-day-old brains; shock effects and all interactions were not significant ($F < 1$). Meningeal MCs were significantly reduced in the

[2] Contrary to formalin fixation, EFA fixation for 48 hr allows optimal maintenance of brain section integrity, clear metachromasia, and excellent cytoarchitectural detail.

TABLE 1
Means and Standard Deviations for Mean Number of MCs per 10-μm Section in the Leptomeninges around the Diencephalon, the Diencephalic Parenchyma, and Combined Measures, According to Body Marking, Foot Shock, and Age Conditions

Mast cells	Grid shock		Body marking		Age	
	Shock (n = 8)	No shock (n = 8)	Marked (n = 8)	Not marked (n = 8)	15 Days (n = 8)	20 Days (n = 8)
Parenchymal						
$\bar{X}$	13.9	9.7	6.2[a,*]	17.3[c]	7.4[a]	16.1[b]
SD (±)	9.9	8.3	5.9	8.5	7.4	8.9
Leptomeningeal						
$\bar{X}$	24.3	23.8	18.2[a]	29.9[d]	33.8[a]	14.3[d]
SD (±)	14.2	13.2	9.1	14.8	11.7	5.0
Total						
$\bar{X}$	38.2	33.5	24.4[a]	47.2[c]	41.2[a]	30.4[b]
SD (±)	19.0	13.4	8.2	14.2	18.4	12.1

* a vs b, $P < 0.05$; a vs c. $P < 0.01$; a vs d, $P < .001$.

marked rats relative to the nonmarked rats ($F = 23.21$, $df = 1, 8$; $P < 0.001$), and in the 20-day-old brains relative to the 15-day-old brains ($F = 64.41$, $df = 1, 8$; $P < 0.001$). A marginally significant ($F = 6.47$, $df = 1, 8$; $P = 0.05$) marking by shock interaction was noted and was due primarily to the greater reduction of MCs in the marked, shocked rats relative to shocked, not marked rats. However, shock effects and other interactions were not significant. Total MC numbers were again significantly ($F = 21.25$, $df = 1, 8$; $P = 0.002$) reduced only in the marked rats relative to nonmarked rats. Interestingly, the reduction in total MC numbers with age was only marginally significant ($F = 5.13$, $df = 1, 8$; $P = 0.05$). All other factors were not significant. One-way ANOVAs showed no significant differences in MC numbers between fixatives ($F < 1$) or litters ($F < 1$). Figure 1, which displays MC numbers for 20-day-old rats, depicts the typical distribution of MC numbers through the subcortical regions measured; the numbers on the horizontal axis also represent the distance in millimeters from the bregma for adult rat brains (after Hart, 1976).

These results clearly suggest that body marking rat pups for identification during preweaning development can be associated with significant 50 to 60% reductions in brain MC numbers. Considering the magnitude of the observed effect and the possible predominance of brain histamine in MCs during this period, further investigations to identify the controlling stimuli appear warranted. Whether the effect was due to the interactive role of the mother (e.g., excessive licking of the marked body surface) or to toxic materials in the marker contents remains to be experimentally determined.

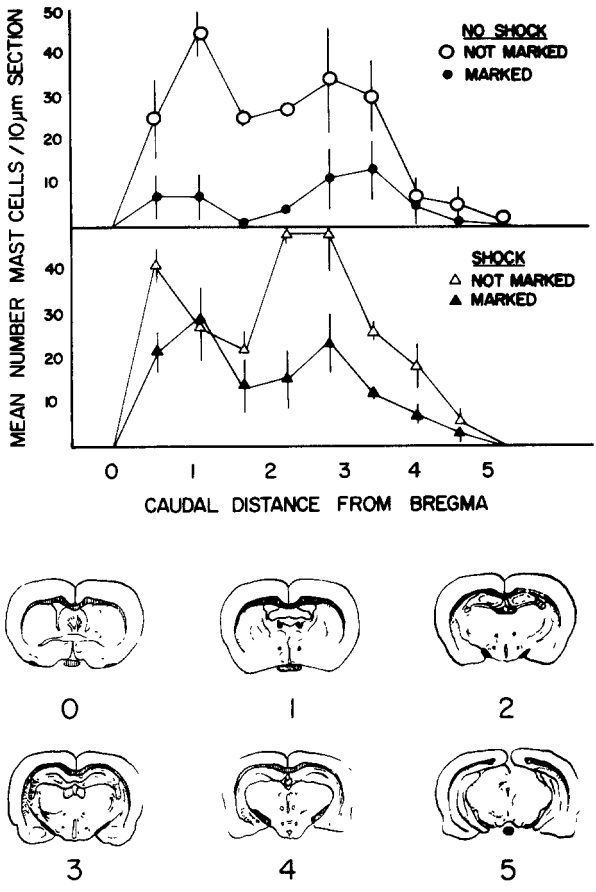

FIG. 1. Mean numbers of brain mast cells per 10-μm section of tissue in subcortical structures at different levels from the bregma, as indicated by sample cross sections (0 to 5), for 20-day-old rats that had received shock (triangles) or no-shock (circles) treatments daily since 5 days of age. Closed symbols (●, ▲) indicate brains from rats that also had been marked daily on their rear dorsal surfaces for identification; open symbols (○, △) indicate brains from similarly handled, but not marked, rats. Vertical lines indicate standard deviations.

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